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GENAPP.002RA PATENT

Applicant	:	Gopal	Group Art Unit: 1636
Reissue Appl.	:	09/404,979	
Filed	:	September 22, 1999	
For	:	PEPTIDE-MEDIATED GENE TRANSFER	
Examiner	:	McKelvey, T.	

Considered
DMG
12/16/02

DECLARATION OF WILLY WRIGGERS, PH.D.

I, Willy Wriggers, Ph.D., do hereby declare and state that:

1. I am a citizen of Germany residing at 5982 Highplace Drive, San Diego, CA 92120.

2. I received my Ph.D. in Physics in 1998 from the University of Illinois at Urbana-Champaign. I am currently an assistant professor at The Scripps Research Institute, Department of Molecular Biology, in La Jolla, California. A true and correct copy of my curriculum vitae is attached.

3. I have extensive educational and research experience in the determination of the 3-dimensional structure of proteins and macromolecular assemblies. In particular, I invented the 'Hingefind' algorithm that searches for structural domains and hinge regions in biomolecular structures. I have been retained by Genetic Applications, the assignee of the above-identified application. I have no ownership interest in the application. Genetic Applications is paying me the normal hourly rate charged by me or my consulting services in this area of my expertise.

4. I understand that the above-identified application is a reissue application of U.S. Patent No. 5,670,347, filed as application Serial No. ("SN") 240,514 on May 11, 1994 ("the Gopal patent"), and that the application has an effective filing date of May 11, 1994.

5. I have reviewed the above-identified application, together with the presently pending claims. As I understand it, the subject matter claimed in the application is directed to a transfection vector comprising a synthetic polypeptide linked electrostatically to a DNA structural sequence, wherein the polypeptide comprises (A) a polymeric chain of basic amino acid residues, (B) a nuclear localization signal (NLS) peptide and (C) a hinge region of neutral amino acids that connects the polymeric chain and the NLS peptide.

6. I am aware of the discussions between the Examiner and the Applicant with regard to U.S. Patent No. 5,994,109 to Woo et al. ("the '109 patent") during prosecution of the above-identified application. In essence, the Applicant was able to overcome any potential rejection over the '109 patent by distinguishing the inventive "hinge region" of the above-identified application from the "spacer" employed in the '109 patent. This is evidenced by an Examiner Interview Summary Record dated December 19, 2000, an Examiner Interview Summary Record dated January 22, 2001, and the fact that the Examiner allowed the application.

7. I understand that Woo et al. obtained additional patents related to the '109 patent, including U.S. Patent No. 6,150,168 ("the '168 patent"). I understand that the '168 patent, which like the '109 patent, is a divisional of application SN 08/167,641, has the same disclosure as the '109 patent. I also understand that the '168 patent contains claims to "a hinge region," including claims to "a hinge region... comprised of glycine and serine." *See* the '109 patent at claims 36, 37, 39 and 40.

8. I understand that both of the above-mentioned Woo et al. patents stem from application SN 07/855,389, filed March 20, 1992, and that therefore the earliest possible effective filing date is March 20, 1992. I have been retained to attest to the state of the art at this time and at the effective filing date of the Gopal patent.

9. Based on my extensive review of the literature, it is clear that the term "hinge region" and the term "spacer" were well defined and used in a mutually exclusive manner by those skilled in the art as of the effective filing dates of the Woo et al. and the Gopal patents. Below, I describe the accepted definitions for the terms "hinge region" and the term "spacer" and show how these terms are used in a mutually exclusive manner. I also demonstrate that the use of these terms in the Gopal patent and the Woo et al. patents is consistent with the well-known definitions.

10. The term "hinge" originated in the field of structural biology. The concept of "hinge-bending, whereby the relative flexibility of short regions of the polypeptide chain allows significant movement of structural [...] domains" (Dobson, 1990) gained widespread acceptance, as a term of art in the 1980s and early 1990s, after evidence for conformational transitions in identical or homologous proteins became known. The distinguishing property of a hinge is its ability to permit large movements of one attached moiety relative to another attached moiety.

11. The term "spacer" had its beginnings in the chemical/biochemical conjugation art as well as the evolutionary biology field. Its underlying meaning is essentially the same in both chemistry and biology: *i.e.*, a spacer acts as a distance holder between two attached moieties. In the biomedical field, for example, spacers are often used to conjugate antigenic moieties to carriers. U.S. Patent No. 4,691,006 to Stevens ("the '006 patent") discloses the use of a peptide spacer to attach an antigenic peptide to a carrier macromolecule. The '006 patent teaches that these spacers "serve to position the sequence which follows physically distant from the carrier-modifier." Spacers are used in several applications to separate attached moieties. For example, preventing a hapten from coming into contact with a carrier molecule or keeping a ligand away from the matrix of an affinity chromatography column. Biologists also describe spacers as being distance holders. Spacers are described in Doolittle & Blombäck (1964), "as those amino acids which only take up room in the peptide chain so that some critical amino acid is maintained in a certain position." (*See* p. 151).

12. The technical use of the terms spacer and hinge had become so common by the time of the Gopal filing that they can be found in widely used college textbooks, such as The Molecular Biology of the Cell (Garland Pub. 3rd Ed., 1994). Actin-binding proteins are described as having spacers of varying lengths 14 nm to 200 nm, resulting in "greater separation between the filaments." (*Ibid.* p.837) The spacers are described as being built in modular fashion from repeating units. (*see* Figure 16-68 legend p. 837). Whereas hinges designate flexible region which allow attached domains freedom of movement. (*see* Figure 15-12 A p. 729).

13. A number of authors have commented on the property of hinge regions to remove steric constraints from the relative motion of the attached domains. Harrison (1980) discloses a hinge region in the tomato bushy stunt virus (TBSV) protein that solves the problem of packing 180 identical proteins into the 60-fold icosahedral symmetry of the virus shell. (*See* pp. 141-144). The functional role of hinges to provide large movements between attached domains is in direct contrast to spacers that are intended to keep the domains apart. Note a clear distinction is made in the art between spacers and hinges by Harrison describing the conformational change in TBSV as hinge rotation (*see* pp. 141-143) in contrast to the separation of interfaces as maintained by spacers (*see* p. 145).

14. "Hinge-bending and folding" is essential to protein domain motion. Dobson compares the structure of "open and closed forms" of proteins to demonstrate hinge-bending motion. (Dobson, p.199) It is clear that domain "closure" (in hinges) is functionally opposite to domain "separation" (in spacers).. Therefore the category "spacer" cannot logically include hinges, since the category "hinges" includes those structures that permit domain closure. Spacers and hinges are, by definition, mutually exclusive.

15. The structures and functions of spacers and hinges are specific and mutually exclusive: spacers act as a distance holder between two attached moieties, while hinges are highly

flexible regions that permit large movements of one attached moiety relative to another attached moiety. Spacers cannot serve as hinges because of the very spatial constraints that permit them to maintain attached moieties in a certain position. Hinges cannot serve as spacers to maintain attached moieties in a certain position because they are free to assume any of a wide range of distances between their attached moieties.

16. Thus, spacers and hinges are used in a mutually exclusive manner. Such specific and opposing definitions cannot be construed as coextensive; as such an exercise would make the terms meaningless.

17. The peptide $[(gly)_i(ser)_j]_k$, wherein i=1-6; j=1-6; and k=3-20, discussed in the Woo et al. patents is a spacer, not a hinge region. This is demonstrated by Huston, which like the Woo et al. patents, discloses a repeating motif of at least three glycine and serine subunits. The stiffness imposed by the repeating motif is in agreement with the analysis of Huston, which discloses that replacement of the linker ($[(gly)_4(ser)_1]_3$) with more rigid linkers does not significantly effect the immunotoxin activity under study. (*See* p. 54). Such repeating motifs are generally recognized in the art as evidence of stiffness: "Any given structure, for instance an oligopeptide, that is repeated, each monomer having the same relationship to its predecessor, will generate a helix." (Matsushima (1990) p. 129). That is, $[(gly)_i(ser)_j]_k$, wherein i=1-6; j=1-6; and k=3-20, would form a stiffened secondary structure. The repeating motif of glycine and serine, in particular, is found in several molecules, *e.g.* RNA-binding proteins. A repeating motif of glycine and other non-glycine residues, such as that disclosed in the Woo et al. patent, is clearly structurally and functionally distinct from a stretch of glycines such as that disclosed in the Gopal patent. As taught in Birney (1993), repeated domains are distinct from segments made up of only consecutive glycine residues, which have been termed glycine hinges, because of their flexibility. (*See* p. 5813).

18. U.S. Patent No. 5,482,858 to Huston ("the '858 patent") states: "As noted above, the spacer's primary function is to separate the active protein regions." (*See* col. 12). A hinge would not ensure that the domains are separated due to it's intrinsic flexibility. Thus the repeating motif of $[(gly)_i(ser)_j]_k$, wherein i=1-6; j=1-6; and k=3-20, has been defined as a spacer in the art.

19. Further, the art taught away from the use of a stretch of glycines such as that employed in the instant application. *See, e.g.,* Argos, which taught that "all-glycine" molecules were "too flexible" and unstable. (*See* p. 956).

20. The disclosure of the Gopal patent (and thus the disclosure of the instant application) consistently uses the specific term "hinge region." The Gopal patent's use of the term "hinge region" is consistent with the above definition and the remaining disclosure, *i.e.*, a region comprising a stretch of small amino acid residues without bulky side groups, *e.g.*, glycine. This hinge region serves to minimize steric interference due to its intrinsic flexibility.

21. In contrast, the Woo et al. patents consistently use the specific term "spacer." All of the examples characterized by Woo et al. as spacers fall within the accepted definition of spacer.

22. The accepted meaning of the terms "hinge" and "spacer" as used by those skilled in the art reveals that both "hinge" and "spacer" have well defined and mutually exclusive meanings. Hinge regions provide flexibility between protein domains (*e.g.*, using a stretch of glycine residues). Conversely, spacers act as a "distance holder" between two moieties. One skilled in the art reading the Woo et al. patents would understand that the use of the word "spacer" has a special meaning. Indeed, it would pointedly exclude the use of a hinge as described in the Gopal patent.

23. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that

such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Respectfully submitted,

Dated: 8/30/02

By: Willy Wrijgers

Willy Wriggers, Ph.D.